

# LABELING TECHNIQUES IN DIAGNOSIS OF ENTEROVIRUS INFECTION<sup>1</sup>

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## INTRODUCTION

Coons (3) has given a succinct account of the principles underlying the use of labeling techniques in the diagnosis of viral diseases, and has given several examples of the application of the same methods to research problems. Schaeffer (11) has clearly shown the usefulness of the technique in the diagnosis of rabies and the differentiation of variola and varicella.

Another area in which labeling techniques may be of great value is that dealing with human enteroviruses and their possible role in the pathogenesis of intestinal infections. These agents, well recognized as transient inhabitants of the alimentary tract, are characterized by small particle size (25 to 30 mμ in diameter), ribonucleic acid core, ether resistance, and cationic stabilization (2). More than 60 distinct antigenic types have been recognized; these are usually classified into four well-recognized subgroups—polioviruses, Cocksackie A viruses, Cocksackie B viruses, and ECHO viruses. Their presence in the gut has suggested an etiological role in diarrheal disease, and has raised the need for the development of rapid identification techniques. The use of labeled antibody is one possible method.

## DIAGNOSTIC PROCEDURES

It is well known that attempts to apply immunofluorescent techniques to the rapid diagnosis of intestinal infections caused by gram-negative bacteria have been unsuccessful to date, owing chiefly to the presence of common antigenic components among the members of the family Enterobacteriaceae which give rise to

interfering cross-reactions. The situation is more favorable in the case of the human enteroviruses, which recently have been classified as a subgroup of the Picornavirus group (6). These agents (with the exception of certain members of the Cocksackie A subgroup) may be isolated and propagated with relative ease in simple tissue-culture systems, and their presence may be recognized by the typical cytopathogenic effects they produce. Significant immunological cross-reactivity has been observed in but few instances and, thus, the identification of these agents on the basis of their antigenic characteristics is a practicable but laborious and time-consuming procedure. The use of labeled specific antisera for the identification of these agents provides a simpler and far more expeditious technique as compared with the neutralization test—the technique currently in use as the standard procedure.

At least three reports during the past 3 years have demonstrated the potential advantages of this approach. Shaw and his collaborators (12) utilized pooled fluorescein-conjugated antiserum pools against Cocksackie and ECHO viruses to make a rapid presumptive identification of these agents. Page and Stulberg (8), using a similar technique with a modification of the same antiserum combination pool scheme originally devised by Lim and Benyesh-Melnick (7), were able to detect and type ECHO, Cocksackie, and polioviruses in spinal fluids or in stools, or both. Similarly, Brown (1) applied this technique for the detection and identification of enteroviruses in stool specimens. He was successful in identifying ECHO and Cocksackie viruses (but not polioviruses) in tissue sections obtained at autopsy. In addition to this use of the direct method of fluorescent microscopy in identifying viral isolates, Brown (1) successfully employed the indirect method for the identification and titration

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of serum antibodies against specific members of the enterovirus group, thus permitting a retrospective diagnosis of viral infection.

#### POTENTIAL APPLICATION TO STUDIES OF PATHOGENESIS OF ENTEROVIRUS INFECTIONS

The mere finding of an organism in the intestinal tract during a bout of diarrheal disease does not itself give conclusive evidence of a significant etiological relationship. Some enteroviruses are undoubtedly mere commensal organisms, rarely if ever giving rise to signs and symptoms of illness. Others produce their characteristic lesions in organs other than the gut, as in the case of the polioviruses. Still a third group, exemplified by certain ECHO viruses, may in one instance cause diarrheal disease and, in another, manifestations of central nervous system involvement. These differing patterns make it difficult to assess the role of the viral agent isolated from the intestine in the pathogenesis of the disease observed (9).

The use of immunofluorescence, and of other labeling techniques (10), offers a possible approach to the solution of the problems with regard to intestinal infections. By use of the peroral biopsy instrument of Crosby (4), it is possible to obtain samples of the gut wall from various parts of the intestine for pathological and microbial studies. This technique has been used with significant results in cholera studies (5), where it has yielded valuable information on the pathological physiology of that disease. A similar approach to the study of intestinal infections presumed due to an enterovirus might yield equally important findings. The samples of intestinal mucosa thus obtained could be examined for the presence of specific viral agents, and the type of cell infected could be determined. Furthermore, the location of the virus in relation to the cell itself (on the surface, intracytoplasmic, intranuclear, etc.) could be studied as well. These findings, when correlated with those obtained by classical histological techniques, might yield

pertinent information on the role of these agents in the pathogenesis of the disease observed.

#### LITERATURE CITED

1. BROWN, G. C. 1963. Fluorescent antibody techniques for the diagnosis of enteric infections. *Arch. Ges. Virusforsch.* **13**:30-34.
2. COMMITTEE ON ENTEROVIRUSES. 1962. Classification of human enteroviruses. *Virology* **16**:501-504.
3. COONS, A. H. 1964. Labeling techniques in the diagnosis of viral diseases. *Bacteriol. Rev.* **28**: 397-399.
4. CROSBY, W. H., AND H. W. KUGLER. 1957. Intraluminal biopsy of the small intestine. *Am. J. Digest. Diseases* **2**:236-241.
5. GANGAROSA, E. J., W. R. BEISEL, CHANYO BENYAJATI, H. SPRINZ, AND P. PIYARATN. 1960. The nature of the gastrointestinal lesion in Asiatic cholera and its relation to pathogenesis: a biopsy study. *Am. J. Trop. Med. Hyg.* **9**:125-135.
6. INTERNATIONAL ENTEROVIRUS STUDY GROUP. 1963. Picornavirus group. *Virology* **19**:114-116.
7. LIM, K. A., AND M. BENYESH-MELNICK. 1960. Typing of viruses by combinations of anti-serum pools. Application to typing of enteroviruses (Coxsackie and ECHO). *J. Immunol.* **84**:309-317.
8. PAGE, R. H., AND C. S. STULBERG. 1962. Rapid identification of enterovirus infections by immunofluorescence. *Bacteriol. Proc.*, p. 137.
9. SABIN, A. B. 1963. Cause and control of fatal infantile diarrheal diseases. *Am. J. Trop. Med. Hyg.* **12**:556-566.
10. SAXEN, L., AND T. VAINIO. 1964. Tetracyclin fluorescence as a marker for the detection of viral lesions *in vitro*. *Nature* **201**:936-937.
11. SCHAEFFER, M., E. V. ORSI, AND D. WIDELock. 1964. Applications of immunofluorescence in public health virology. *Bacteriol. Rev.* **28**:402-408.
12. SHAW, E. D., A. NEWTON, A. W. POWELL, AND C. J. FRIDAY. 1961. Fluorescent antigen-antibody reactions in Coxsackie and ECHO enteroviruses. *Virology* **15**:208-210.